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CHARACTERIZATION OF THE XRCC2 HUMAN DNA REPAIR PROTEIN;

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XRCC2 is a human repair protein that corrects the hypersensitivity of hamster mutant irs1 to ionizing radiation, cross-linking agents, and other DNA damage agents. The *XRCC2* cDNA, encoding at least 280 amino acids, was isolated using functional complementation. The *XRCC2* genomic DNA, obtained by screening a P1 library with *XRCC2* cDNA probe (GenomeSystems, Inc.), also complemented irs1 but was found to be truncated at the 5' end and missing ~80 bp in the cDNA. The *XRCC2* cDNA and genomic DNA transfor-mants showed partial correction in terms of cell survival for MMC, but showed nearly full correction for cisplatin as examined by a differential cytotoxicity assay. With ionizing radiation, the genomic transformant showed wild-type sensitivity while the cDNA trans-formants surprisingly showed no correction. Chromosomal aberrations showed intermediate correction in both plasmid and genomic transformants in unirradiated cultures as well as after 100 cGy of ^{137}Cs γ-rays. The predicted protein sequence shows weak similarity with *S. cerevisiae* Rad51 and contains consensus ATP binding domains. The *XRCC2* cDNA was cloned into expression vectors pET32 and pET26 (Novagen) and overexpressed in *E. coli.* His-tagged thioredoxin(trx)-XRCC2 fusion protein was obtained in the soluble fraction and purified to near homogeneity using a Ni-NTA agarose column (Qiagen). Insoluble pelB-XRCC2 fusion protein with C-terminal His-tag was purified in denaturing 8 M urea, gradually dialyzed to 1 M urea, and used for producing polyclonal antibodies in mice. The antisera detected both the overexpressed pelB-XRCC2 and the trx-XRCC2 fusion proteins, and they also detected a protein of ~ 50 kDa in HeLa cell extract. Thus, the current *XRCC2* cDNA is probably truncated at the 5'-end. Experiments are in progress to obtain the cDNA sequence upstream of the existing cDNA as well as the missing portion of the genomic sequence.
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